IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	McDonald *et al.*	Group Art Unit:	1647
Serial No.:	09/360,242	Examiner:	Landsman, R.
Filed:	July 22, 1999		

For: *METHODS AND COMPOSITIONS FOR TREATING SECONDARY TISSUE DAMAGE AND OTHER INFLAMMATORY CONDITIONS AND DISORDERS*

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
Alexandria, VA 22313-1450

Sir:

I, JOHN R. McDONALD, declare as follows:

1. I am an inventor of and am familiar with the subject matter of the above-captioned application.

2. I received B.Sc. and Ph.D. degrees at Napier College, Edinburgh, completed successful postdoctoral appointments in Canada and The United States before leaving academia for the biotechnology industry (Boulder CO, and San Diego CA). I have been involved in all aspects of the Research and Development process from project planning through IND filing. My research has focused upon growth factor signal transduction, multiple sclerosis, and the purification and characterization of neurotrophic factors and growth factor-mitotoxin fusion proteins. I have received several peer-reviewed awards and grants, including a US National Institutes of Health Small Business Innovation Research Grant. I am co-author of over fifty publications, and a named inventor on nine patent applications.

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I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to: Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA, 22313-1450.

Stephanie Seidman

3. I am a founder of Osprey Pharmaceuticals Limited, Canada. I was Vice-President of Research & Development and a Director at the company from its founding in 1997 to 2002 Since 2005, I have held the position of Director of Science and Intellectual Property at the company.

4. As described in previous DECLARATIONS of record in this application, the conjugates described in the above-captioned application and encoded by the described nucleic acid molecules, are broad-based, widely applicable anti-immunoinflammatory drugs for inhibiting activation, proliferation and/or migration of immune effector cells. As a result of such activity, these conjugates can be used in methods for such inhibition. Activation, proliferation, and/or migration of immune effector cells are known to play a role in the etiology and/or pathology of a variety of diseases and conditions. Numerous such diseases and conditions and the role of immune effector cells in each are described in the application. These diseases and conditions, include secondary tissue damage-associated disorders such as those that accompany central nervous system trauma and disease, including spinal cord injury and head injury, auto immune diseases, including multiple sclerosis, amongst others, and other inflammation-driven diseases as divergent as asthma, arthritis, HIV and cancer.

5. In my capacity as a Director, I directed the experiments described below **(Section B)**. These experiments demonstrate the effectiveness of conjugates of chemokine receptor-targeting agents for treatment of diseases that are characterized or caused by a pathophysiological inflammatory response. These data are in addition to data already of record. These data and the data of record further demonstrate that these conjugates provide a more selective and targeted delivery than previous ligand-directed delivery conjugates. The data indicate that the chemokine-toxin conjugates target cells with specificity and a certain degree of predictability. In addition, the data indicates that these agents distinguish between activated and quiescent cells and that potential toxic side effects may be a non-issue or at most, minimal.

6. This DECLARATION also provides a discussion of the inflammatory disease process and provides citations to numerous articles published before the July 21, 1998, priority date of this application **(Section A)**. This discussion is provided to describe and establish that the role of immune effector cells in the inflammatory response was well known at the time of filing of this application, to establish that treatments targeting immune effector cells are known to be effective, and to rebut scientifically unsound or incorrect assertions in the Office Action. Attention also is directed to the application, which also includes such

disclosure. As described at length in the application and discussed at length in previous responses of record, the instant application provides is a new modality for targeting immune effector cells. As established during the lengthy prosecution of this application, there is no disclosure, teaching or suggestion in the art for the preparation of conjugates that target chemokine receptors. Inhibiting and depleting immune effector cells for treatment of diverse diseases, however, has been used for at least more than 50 years; the instant application provides a new way to achieve this result. Examples of such treatments to demonstrate that inhibiting and/or depleting immune cells are effective and are discussed below in **SECTION C.**

7. Also, provided in this DECLARATION is a summary of recent developments in the field that support the operability of the methods in this application and validate the approach as it was described in the instant application and its parent applications **(SECTION C)**. Any literature published after the priority date of the application is cited in order to provide such evidence of operability and validation of the generic approach provided by the application.

Section A. The Inflammatory Response

1. The immune system

The immune system can be divided into the innate and adaptive arms that together confer a host defense system comprised of different immune effector cells. The innate immune system relies on cells immediately reactive toward invading entities, such as microbes, and includes phagocytosing macrophages, neutrophils (polymorphonuclear neutrophils, PMN) and natural killer T cells (NK). The adaptive immune system includes T and B cells, which require activation by antigen presenting cells of the innate immune system in order to target specific host invaders. Cells of the innate and adaptive immune responses work in concert with tissue residential cells (TRC) in order to maintain a homeostatic balance in many organ specific processes including embryogenesis, angiogenesis, lymphocyte trafficking, wound healing, tissue repair, removal of cellular debris and other unwanted agents such as microbes, viruses or cancer cell clones. Macrophages, monocytes, and microglia (collectively referred to as mononuclear phagocytes, MNP), PMN, eosinophils, subtypes of the T-lymphocyte family and other immune cells are responsible for these homeostatic processes and for the maintenance of an intact surveillance and host defense system.

The generic inflammatory response is a multi-factorial biochemical process that is orchestrated and perpetuated by cells of immune effector cell lineage. Soluble factors released from injured and dying cells, immune complexes or complex charged antigens like bacterial lipopolysaccharides (LPS) and viral envelope proteins working via the complement and toll receptor system are common triggers of leukocyte activation and recruitment. In response leukocytes undergo profound phenotypic changes including the upregulation of cell adhesion molecules (CAM) and proinflammatory cytokines and chemokines for trafficking and communication with other leukocyte groups.

The recruitment of leukocytes from the blood to specific tissues in homeostatic or inflammatory environments is highly regulated and orchestrated by chemokines, a variety

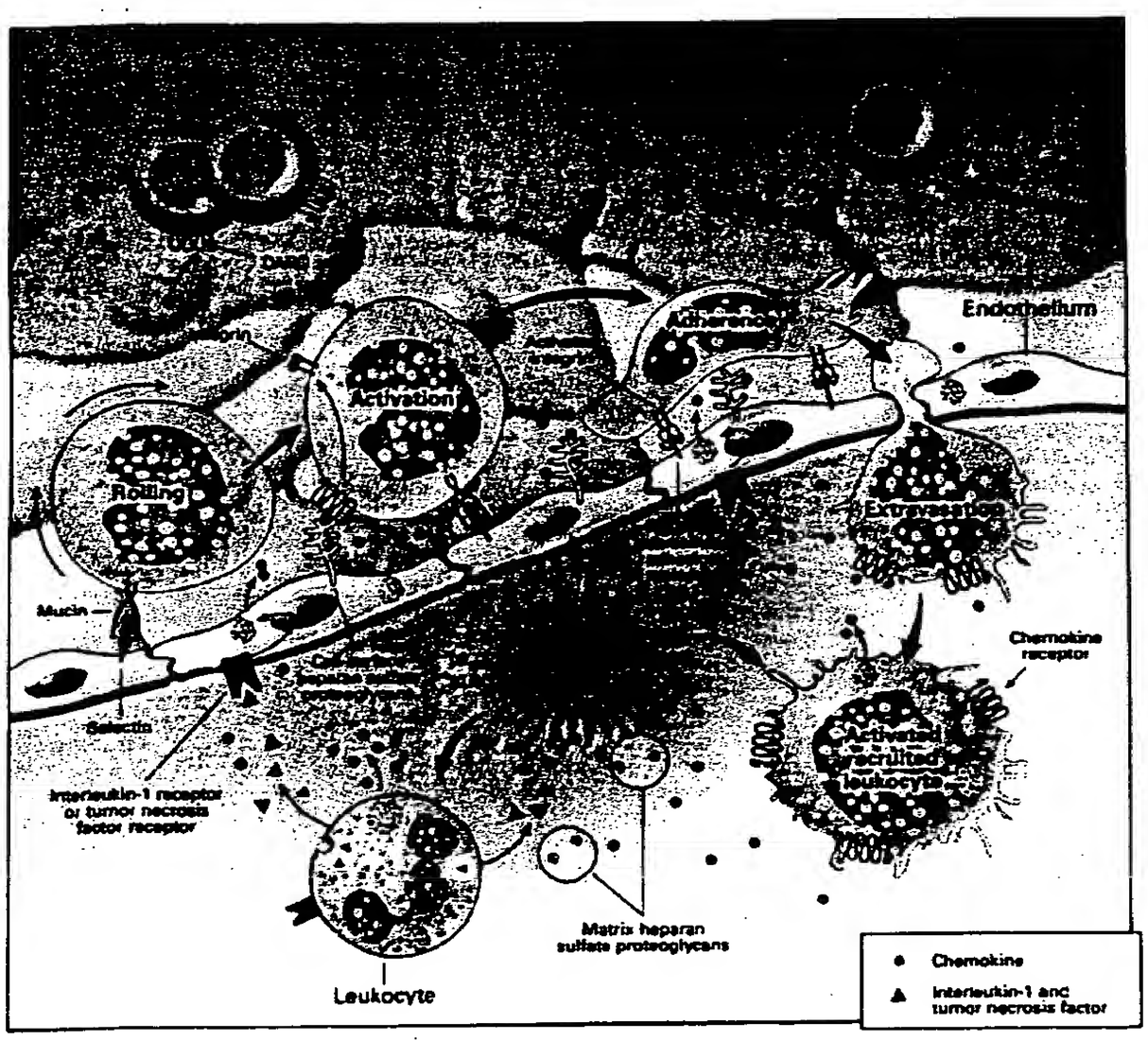


Diagram 1. Trafficking of Leukocytes: In response to chemokine gradients leukocytes traffic to selected tissues in the body and carry out homeostatic (e.g., immunosurveillance) or become involved in disease pathology. Rolling, activation, adherence and extravasation (movement form blood vessels in to tissue) occur in a sequential manner. Rolling occurs in response to chemokines and therefore the receptors are expressed. Thus delivery of a claimed chemokine-toxin will kill cells prior to pathological activation and thereafter. (see, *e.g.*, Tanaka *et al.*(1996) *J. Exp. Med. 184*:1987-1997; Carlos *et al.*, (1994) *Blood 84*:2068-210; Alon *et al.* (1994) *The J. Cell Biol. 127*:1485-1495)

of CAM, immunoglobulin CAM, and glycosaminoglycans (GAG). The detailed nature of specific molecular migratory patterns differs among leukocyte subtypes, the tissue of destination and the type of migratory stimuli. In general, leukocytes migrate toward a gradient of adherent chemokines along the endothelial cell walls. The gradient is formed by

chemokine attachment to GAG (e.g., heparin sulfate) and promotes presentation of the ligand(s) to receptors on the traveling leukocytes. The extravasation of cells into the tissues occurs in several steps; tethering, rolling, activation, firm adhesion and extravasation (Diagram 1; Tanaka *et al.*(1996) *J. Exp. Med. 184*:1987-1997; Carlos *et al.*, (1994) *Blood 84*:2068-210; Alon *et al.* (1994) *The J. Cell Biol. 127*:1485-1495). All steps involve the binding of leukocytes to the endothelium by families of CAM expressed on both sets of cells. Tethering begins with weak interaction of leukocytes to the endothelium promoted by selectins. Migratory cells begin to roll and adhesion to other selectins enhances the tethering strength and stabilizes cell rolling. Activation and adherence is mediated by leukocyte expression of integrins and immunoglobulin CAM. The integrins bind firmly to their ligands on the endothelial cell, selectin is shed by leukocytes and rolling ceases. Chemokines and integrin binding activate the cells and leukocytes are ready to traverse the endothelium into the tissue.

The cells also produce and secrete proteases that degrade basement membrane and extracellular matrix (ECM) components, thus aiding entry into and migration within the tissue. The expression of specific chemokines, receptors and CAM contribute to the selective locomotion and tissue specificity of leukocyte subgroups. Once at the site of injury (or disease) leukocytes produce an armament of cytotoxic mediators (see, *e.g.*, Benveniste (1997) *J. Mol. Med. 75*:165-173; Stout *et al.* (1997) *Frontiers in Bioscience 2*:197-206). Reactive oxygen and nitrogen species, proteolytic enzymes and eicosanoids kill off invading microbes and fungi which are phagocytosed principally by MNP and PMN. Leukocyte (especially macrophage) derived growth factors (GF) including vascular endothelial growth factor (VEGF) and fibroblast GF (FGF) facilitates angiogenesis. Angiogenesis helps in tissue repair but can also contribute to pathological conditions.

2. Immune effector cells leukocytes underlie or are involved in the pathogenesis of a variety of diseases and disorders

As described in the application immune effector cells are involved in the pathogenesis of a variety of diseases and disorders. As described in the application immune effector cells refer to all cells involved in innate and adaptive immunity and include leukocytes and lymphocytes both which can be broken down again into antigen presenting cells and granulocytes (e.g., neutrophils, eosinophils and mast cells). The descriptions and classifications of cell types overlap greatly. In any pathology a mixture of these cell types are involved. As described in the application, an over-zealous infiltration (migration), (chronic)

activation and proliferation (increased numbers) of relatively disease-specific subtypes of leukocytes are responsible the inflammatory response that leads to tissue destruction in the pathology of a wide range diseases. Numerous diseases and conditions and disorders, such as secondary tissue damage including spinal cord injury (SCI), autoimmune diseases including multiple sclerosis, inflammatory disease, such as inflammatory bowel diseases and inflammatory joint diseases, allergic diseases, inflammatory lung diseases, CNS diseases including Alzheimer's Disease, traumatic brain injury, stroke, and other neurodegenerative diseases, inflammation after gene therapy, and cancers, and the role of the inflammatory response and the chemokine system are described in the application. The application describes, as exemplary, the role of leukocytes in each such disease and chemokines and receptors therefore in each such disease.

Table 1, below, lists only some of about 200 diseases in which immune effector cells, including, activated leukocytes play a role. This Table was compiled from about 150 review articles; with the focus to provide information known at the time of the filing of earliest priority application. References that provide such information include art cited throughout the specification and made of record in the application. Other references include: Makita *et al.* (1998) *Am. J. Respir. Crit. Care Med. 158*:573-579; Kaartinen *et al.* (1995) *Arterioscler. Thromb. Vasc. Biology 15*:2047-2054; Kaartinen *et al.* (1996) *Circulation 94*:2787-2792; Barnes *et al.* (1998) *J. Clin. Invest.101*:2910-2919; Qin *et al.* (1997) *J. Clin. Invest. 101*:746-754; Ogata *et al.* (1997) *J. Pathology 182*:106-114; Ying *et al.* (1997) *Eur. J. Immunol. 27*:3507-3516; Gauvreau *et al.* (1997) *Am. J. Respir. Crit. Care Med. 156*:1738-1745; Ponath *et al.* (1996) *J. Clin. Invest. 97*:604-612; Gonazalo *et al.* (1996) *J. Clin. Invest. 98*:2332-2345; Desbaillets *et al.* (1997) *J. Exp. Med. 186*:1201-1212; Youngs *et al.* (1997) *Int. J. Cancer 71*:257-266; Leek *et al.* (1997) *Cancer Res. 56*:4625-4629; Pantoni *et al.* (1998) *Arterioscler. Thromb. Vasc. Biology 18*:503-513; Sansores *et al.* (1997) *Ches. 112*:214-219 ; Pawluczyk *et al.* (1997) *J. Am. Soc. Nephrology 8* :1525-1536; Zoja *et al.* (1997) *J. Am. Soc. Nephrology 8*:720-729; Lavaud *et al.* (1996) *J. Am. Soc. Nephrology 7*:2604-2615; Rastaldi *et al.* (1996) *J. Am. Soc. Nephrology 7*:2419-2427; Wada *et al.* (1996) *FASEB J. 10*:1418-1425; Hvas *et al.* (1997) *Scand. J. Immunol. 46*:195-203 ; Huitinga *et al.* (1995) *Clin Exp Immunol 100*: 344-51; Gerriste *et al.* (1996) *Proc Natl Acad Sci 93*: 2499-504; Chiang *et al.* (1996) *J. Clin. Invest.* 97:1512-1524; Matsumura *et al.* (1996) *J. Clin. Invest.* 97:2192-2203; Zwacka *et al.* (1997) *J. Clin. Invest. 100*:279-289; and Teixeira *et al.* (1997) *J. Clin. Invest. 100*:1657-1666). These references describe leukocyte-mediated

diseases such as acute lung injury; arthritis; asthma; atherosclerosis; cancers; cerebral ischemia; chronic kidney diseases; chronic obstructive pulmonary disease (COPD); dermatitis; emphysema, encephalomyelitis; HIV-associated diseases (HAD), ischemic/reperfusion injury (*e.g.*, liver and myocardium) and multiple sclerosis. Researchers had shown, for example, that, with the use of fairly limited leukocyte-depleting reagents, the pathologies of experimental allergic encephalomyelitis (an multiple sclerosis model) and a colitis model can be ameliorated (see, *e.g.*, Giulian, D.(1987) *J. Neurosci. Res.18*:155-171; Giulian *et al.* (1993) *J. Neurosci. 13*:29-37; Giulian *et al.* (1990) *Ann. Neurol. 27*:33-42; Giulian *et al.* (1995) *Neurochem, Int. 27*:119-137; Giulian *et al.* (1989) *J. Neurosci. 9*:4416-429; Giulian *et al.* (1988) *J. Neurosci. 8*:4707-4717; Giulian *et al.* (1988) *J. Neurosci. 8*:2485-2490; Giulian *et al.* (1988) *J. Neurosci. 8*:709-714; Giulian *et al.* (1996) *J. Neurosci. 16*:3139-3153; Giulian *et al.* (1995) *J. Neurosci. 15*:7712-7726; Giulian *et al.* (1994) *Dev. Neurosci.16*:128-136; Giulian *et al.* (1993) *J. Neurosci. Res. 36*:681-693, which are of record in this application). In each case amelioration of the pathological signs were correlated with the decreased numbers of leukocytes (see, *e.g.* Bauer *et al.* (1995) *Glia 15*: 437-46; Huitinga *et al.* (1995) *Clin Exp Immunol 100*: 344-51; Natsui *et al.* (1997) *J Gastroenterol Hepatol. 12*; 801-8). For the diseases, conditions or disorders involving an inflammatory response leading to secondary tissue damage, the secondary tissue damaging response increases the zone and severity of an initial injury.

In pathological situations, TRC including glial cells of the CNS, mesangial cells (MC) of the kidney, endothelial cells of many organs and leukocytes can be activated by a great number of stimuli including viruses, bacteria, parasites, proinflammatory cytokines and chemokines, hypoxia, ischemia, proteinuria (protein in the urine), autoantibodies, systemic nucleotides, complement, immune complexes, immunoglobulins and environmental pollutants such as cigarette smoke. These can be the initiating factor(s) of disease, but TRC and inflammatory leukocytes are the soldiers of disease pathology. Activated TRC and resident leukocytes express among other things members of the cytokine superfamily and several powerful leukocyte chemoattractants of the chemokine superfamily, which facilitate leukocyte activation, infiltration and proliferation at the site of inflammation. Many investigators and clinicians had shown that there is a correlation between numbers and increased activity of leukocytes with the severity of disease and measured pathological parameters (*e.g.*, Wada *et al.* (1996) *FASEB J. 10*:1418-1425; Zoja *et al.* (1997) *J. Am. Soc. Nephrology 8*:720-729; and Chiang *et al.* (1996) *J. Clin. Invest.* 97:1512-1524).

As described in previously and discussed again in **Section C,** killing (depletion) of leukocytes and other immune effector cells or inhibition thereof is an established approach for treatment of such diseases. As discussed previously, the role of activated leukocytes in disease was known; using the chemokine receptor system for targeting such cells for depletion and/or inhibition of proliferation, migration or activation was not known. As described in the application and in previous Declarations of record, we have identified the chemokine system, via targeting of cells that express receptors therefor, as a target for intervention in the inflammatory response.

Table 1. Immune Effector Cell Types in Diverse Human Diseases

DISEASE/TRAUMA	MAJOR LEUKOCYTE SUBTYPES
Cancers (all organs)	
General Growth, Angiogenesis & Metastasis	TAM, T, Eosinophils, B, MaC, PMN, DC, Basophils
Breast Cancer	TAM, CD, T, PMN, B
Glioma	TAM, PMN, DC
Kidney Cancer	TAM, PMN
Ovarian Cancer	MNP, T, NK, MaC
Cardiovascular Diseases	
Atherosclerosis	MNP, T, PMN
Myocardial Infarction	MNP, PMN, T, MaC
Restenosis	MNP, T, Eosinophils
Chronic Kidney Diseases	
Diabetic Nephropathy	MNP, T, PMN, MaC
Glomerulonephritides	MNP, PMN, T, MaC, DC
IgA Nephropathy	MNP, T, PMN, MaC, DC, B
Lupus Nephritis	MNP, T, PMN, B, DC, MaC
CNS Diseases and Trauma	
Alzheimer's Disease	MNP, T, PMN
Multiple Sclerosis	MNP, T, Th1, PMN, B
Traumatic Brain Injury	MNP, T, PMN
Spinal Cord Injury	MNP, T, PMN
Spongiform Encephalopathies	MNP, T, B, DC
Stroke	MNP, T, PMN, DC, MaC
Eye Diseases	
Conjunctivitis	MNP, T, MaC, Eosinophils, B
Proliferative Vitreoretinopathy	MNP, PMN, T
Retinitis and Iritis	MNP, PMN, B, T
Uveitis	MNP, T, PMN, DC
HIV and AIDS	MNP, T, MaC, DC
Inflammatory Bowel Diseases	
Crohn's Disease	DC, T, MNP, B, MaC, Eosinophils, PMN
Ulcerative Colitis	MNP, T, B, DC, Eosinophils, MaC, PMN
Eosinophilic Gastroenteritis	Eosinophils, Th2, MaC, B, PMN
Joint Diseases	
Gout	MNP, PMN, T, Eosinophils
Osteoarthritis	MNP, B, T, PMN, DC
Osteoporosis	MNP, T

Rheumatoid Arthritis	MNP, DC, PMN, B, T
Liver Diseases	MNP, Th1, K, NK, MaC, B, GC
Pulmonary Diseases	
Acute Lung Injury	PMN, MNP, T, MaC
Acute Respiratory Distress Syndrome	PMN, MNP, T, GC, MaC
Asthma	Eosinophils, MNP, B, Th2, MaC, NK
Chronic Obstructive Pulmonary Disease	MNP, T, PMN, DC, MaC, Eosinophils
Cystic Fibrosis	PMN, MNP, Eosinophils, MaC, T, B
Emphysema	MNP, PMN, T, MaC, Eosinophils
Eosinophilic Pneumonia	Eosinophils, MNP, MNP, T, GC
Pulmonary Fibrosis	PMN, T, Eosinophils, MNP, MaC
Skin Diseases	
Dermatitis	MNP, DC, T, MaC, Eosinophils, B, PMN
Eczema	MNP, T, DC, MaC, Basophils
Psoriasis	T, MNP, DC, MaC, Basophils, Eosinophils, PMN
Systemic Diseases	
Behcet's Disease	PMN, T, B, MNP, Basophils, MaC
Sarcoidosis	MNP, PMN, T, Eosinophils, NK, GC
Scleroderma	MNP, T, Eosinophils, MNP, DC, B, Basophils, NK
Sepsis	PMN, MNP, T
Sjogren's Syndrome	T, B, MNP, DC, MaC, PMN
Systemic Lupus Erythematosus	PMN, T, MaC, B, MNP, DC, Basophils
Obesity	MNP, T, MaC, Adipocytes
Transplantation	
Graft Versus Host Disease	MNP, T, DC, MaC, Eosinophils, PMN, B
Graft/Organ Rejection	MNP, T, DC, MaC, Eosinophils, NK, B
Vascular Diseases	
Giant Cell Arteritis	GC, MNP, T, DC
Hypertension	MNP, PMN, T, Basophils
Varicose Veins	MaC, MNP, DC, T
Vasculitides	T, PMN, MNP, Eosinophils, GC

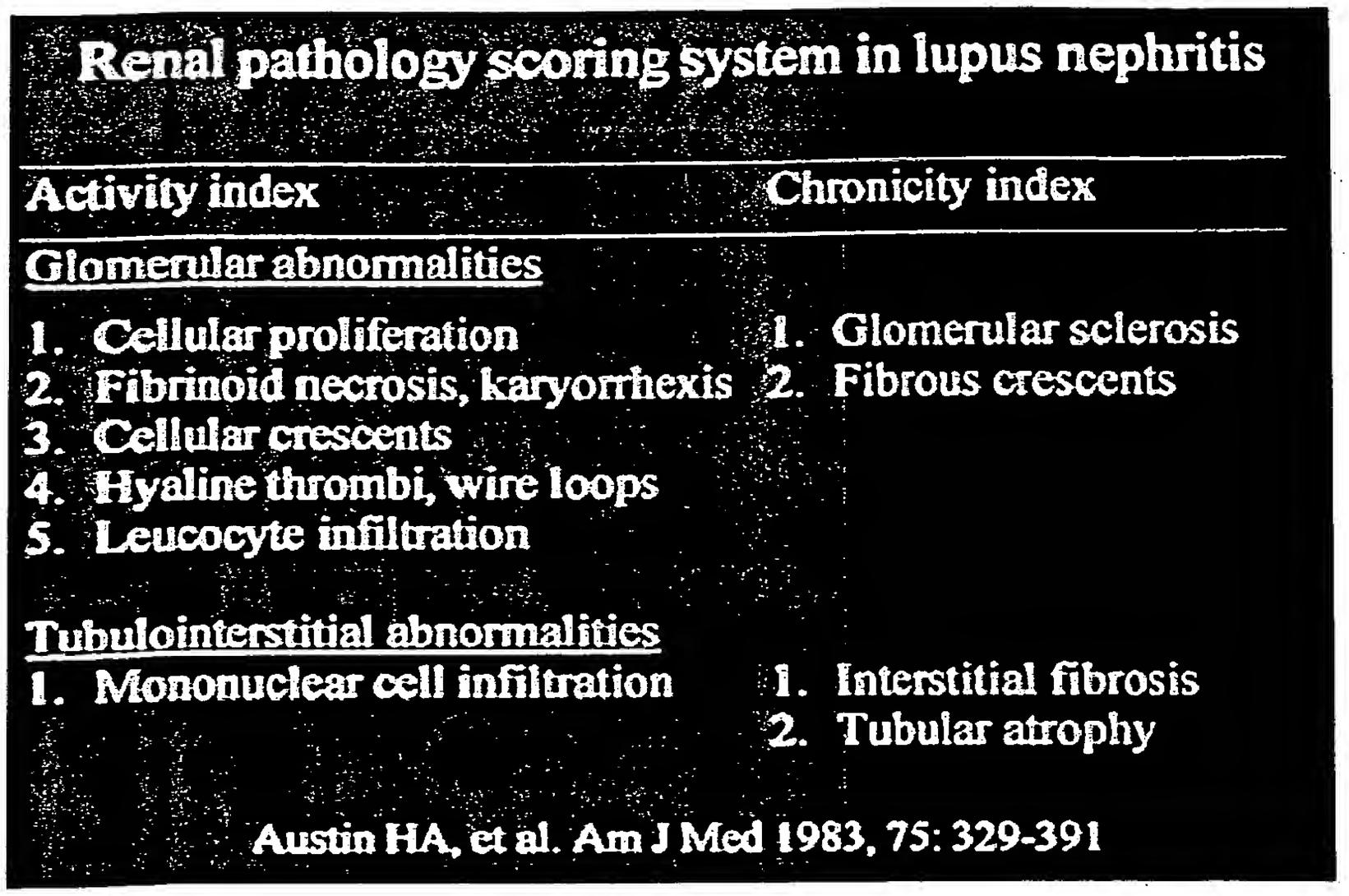
Key: B, B cells; DC, dendritic cells; GC, giant cells (multinucleated fused macrophages); MaC, mast cells; MNP, mononuclear phagocytes (monocytes, macrophages and microglia); PMN, polymononuclear neutrophils; T, various subtypes of T cells; Th2, type 2 helper T cells. N.B. Many disease categories listed here include several distinct diseases. It is important to note that the composition of the microenvironmental milieu of inflammatory factors etc has effects on the phenotypes of different cells. For example, PMN are known to express CXC receptors but in certain cases like septic acute lung injury and reperfusion injury they express CC receptors including CCR2. Under pulmonary diseases above OPL-CCL2-LPM can be used to treat ALI, ARDS, COPD and sepsis as all cell types listed express CCR2.

For selection of a conjugate drug provided in the application, the chemokine receptor targeting agent selected depends upon the immune effector cell(s) involved, the tissue in question and the stage of injury or disease. *But* as is evident from above, *and as described in the application*, particular cells and tissues involved in a particular disease with an underlying inflammatory pathology were known to those of skill in the art at the time of filing and/or can be determined using known methods (see papers cited herein and in the application; see Table 1). The application describes what cells and chemokines are involved in large number of

diseases and also identifies chemokine receptor targeting agents for use to treat such diseases. As shown in Table 1 and the cited references, cell types involved in particular diseases that result from or involve inflammatory responses were known at the time of filing of the priority application. As described in the application, chemokine receptor expression profiles also were known.

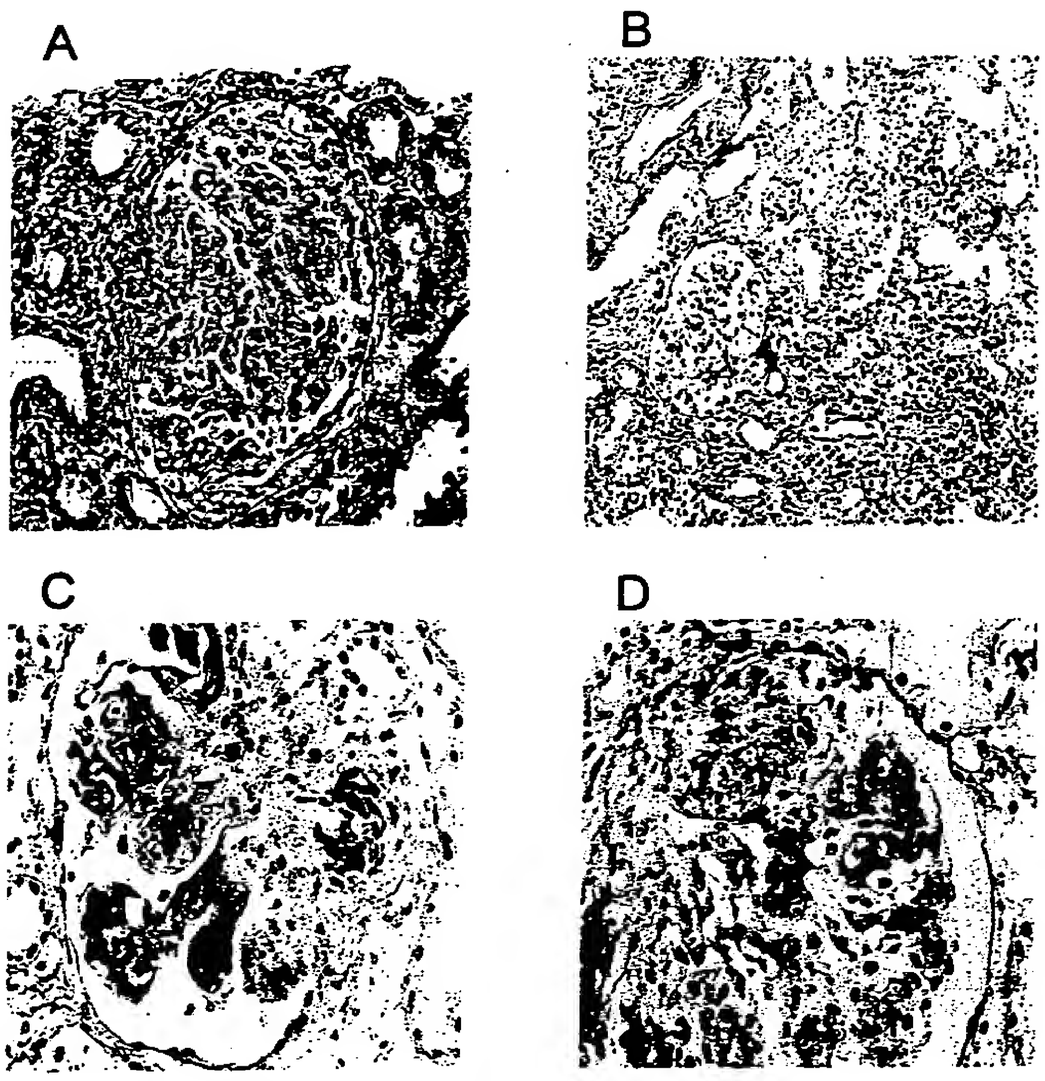
Chemokines perpetuate their own production and are released from immune effector cells, including leukocytes, via autocrine and paracrine mechanisms. They also induce the synthesis and release of cytotoxic compounds from the cells that they target. Resident and infiltrating leukocytes release the very same molecules to mediate tissue damage as used for homeostatic purposes mentioned above (Benveniste (1997) *J. Mol. Med. 75*:165-173; Stout *et al.* (1997) *Frontiers in Bioscience 2*:197-206). Chemokines induce expression of CAM and cell surface antigens (including cytokine and chemokine receptors) on various cell types including leukocytes, endothelial, glomerular mesangial cells and CNS glial cells, thus upregulating the expression of proinflammatory mediators. Therefore, the vicious cycle continues with secondary tissue damage leading to diseased states. This culminates in the destruction of (organ) tissue resident cells, a decrease in organ function and eventually organ shut down. Chronic kidney disease (CKD) is an example of such a disease. CKD patients are routinely treated with immunosuppressive drugs and research has shown that interference with principally macrophage activity slows the progression of disease as measured by the pathology scoring systems (Slide 1) for the specific disease including proteinuria, albuminuria and degree of crescent formation (fibrosis). Figure 1 shows stained slides of human lupus nephritis tissue and the secondary tissue damage that inflammation can orchestrate.

Slide 1



Immune cell infiltration and contribution to fibrosis was appreciated well before 1983.

Figure 1. Histological findings of (A) glomerular crescents/fibrosis (B) interstitial inflammation (mixed groups of immune cells) and (C) immune deposits and (D) PMN infiltration and fibrinoid necrosis. Renal biopsy tissue taken from a 40 year old woman in June 1998 (Ponticelli C. and Moroni G. Division of Nephrology and Dialysis, Maggiore Hospital IRCCS, Milan; http://www.sin-italy.org/jnonline/forum/quattro/case_2.htm).



The references and discussion herein, as well as the application, establish that prior to the filing date of the priority application, it was well established that different dominant immune effector cells are pivotal in the pathogenesis of a variety of diseases. For example, eosinophils, Th2 (CD4+) cells and mast cells (MaC) in asthma; PMN, macrophages and Th1 (CD8+) cells in COPD; MNP and PMN in spinal cord and brain injury: PMN and macrophages in acute lung injury: MNP and Th1 cells in multiple sclerosis; macrophages, Th1 cells and MaC in several CKD: macrophages and T cells in atherosclerosis and granulomatous lung disease; T cells and macrophages in arthritis and several leucocyte types in different cancers were known..

Hence, as described in the instant application, chemokine-regulated leukocyte actions are part of in the immunopathology of diseases in diverse areas of study including: oncology, neurology, nephrology, rheumatology, virology, cardiology, pulmonology, gastroenterology, hepatology, gynecology, dermatology, endocrinology, mycology and transplantology (see Table 1 for examples of diseases that are leukocyte-mediated and the principal leukocyte groups involved; see also, the application Tables 1 and 2, the Examples and sections E and G of the application).

Conclusions

The activities of leukocytes are most exquisitely regulated by the chemokine family of ligands and receptors. Targeting the chemokine system is an elegant way to exploit this exquisite regulation to inhibit migration, activation and/or proliferation of such cells to thereby alter disease progression. There are several levels of regulation that are targeted. The most apparent regulation occurs at the level of the ligands and their receptors; both typically are significantly elevated in response to proinflammatory stimuli. The next area of regulation derives from the distribution of receptors across different cells (leukocytes). Each cell type has a chemokine receptor profile that is akin to a fingerprint or "chemoprint" except that it changes with the specific cell type, tissue type, disease type, function type and is time dependent. Quiescent cells will quickly change and upregulate receptor expression once activated and /or undergoes differentiation. Many chemoprints were known at the time of filing of the priority application.

The identity of a chemoprint also depends on the types and abundance of inflammatory and non-inflammatory mediators in the milieu. Most receptors are inducible in that they are not expressed unless there are inflammatory stimuli. For example, the inducible CCR2 is expressed on macrophages, monocytes, T cells, and basophils and can be targeted by MCP-1/CCL2. Leukocyte subtypes that do not express the prerequisite chemokine receptor or are not physiologically close to areas of inflammation areas are *not activated* and consequently do not participate in inflammatory processes. The eotaxin/CCL11 and CCR3; MCP-1/CCL2 and CCR2; SDF-1/CXCL12 and CXCR4; IP-10/CXCL10 and CXCR3 and IL-8/CXCL8 and CCL3/CCR1 ligand – receptor axes offer good therapeutic targets because of the fidelity of the ligands for their receptors and the fact that they are implicated in several specific diseases.

Therefore, as described in the application, a chemokine receptor targeting agent, and hence a conjugate, can be selected based upon the type of immune effector cell(s) present at a site of inflammation in a particular disease. For example, as described in the application and further demonstrated herein, Eotaxin targets CCR3 expressing eosinophils and Th2 cells in allergic asthma and skin diseases. MCP-1 targets macrophages and Th1 cells in CKD, atherosclerosis, multiple sclerosis and SCI. SDF-1 and MCP-1 target CXCR4 and CCR2 respectively, on specific tumor cells, leukocytes and activated (proliferating) endothelial cells, which are responsible for neovascularization/angiogenesis and tumor nourishment, to

eradicate or slow down the progress of the disease. The experimental results provided herein **(see Section B)** and in previous DECLARATIONs evidence this.

Furthermore, sets of diseases have certain leukocyte profiles *e.g.*, eosinophilic diseases and Th1/macrophage diseases. As a result, the LPMs, as described in the application, can be used in seemingly diverse diseases. These diverse diseases, share the common underlying pathological activation of leukocytes. In addition certain diseases are linked to co-morbidities that have the same pathology. Multiple sclerosis patients have a good chance of developing uveitis (inflammation of the anterior chamber of the eye). There is an old adage for CKD patients stating that they are more likely to die of heart disease than reach end stage kidney failure. Macrophages are pivotal in the disease pathologies in both cases. Obesity begets diabetes begets CKD (due to hyperglycemia). Indeed 40% of all CKD cases are diabetics. Hence these drugs are beneficial by virtue of their activity in targeting chemokine receptors, which express in different pathologies of the same disease condition.

Section B: Studies and results

The conjugates of the instant application, referred to herein as Leukoctye Population Modulators (LPMs), are named using current chemokine nomenclature, which identifies ligands (L) and receptors (R) by their chemokine sub-division followed by a number. For example the ligands monocyte chemoattractant protein (MCP)-1 and stromal-derived cell factor (SDF-1) are referred to as CCL2 and CXCL12, respectively. Their receptors are referred to as CCR2 and CXCR4, respectively. Data demonstrating activity as taught in the application for the following four LPMs is discussed in this DECLARATION:

1. OPL-**CCL2**-LPM (**MCP-1-AM**-ShigaHIS ; formerly designated OPL98110 in Table 6 of the application was used for *in vitro* data and OPL98102 MCP-1-AM-Shiga was used for the *in vivo* data;
2. OPL-**CCL11**-LPM (**Eotaxin –AM**-ShigaHIS; formerly designated OPL98112 in Table 6 of the application);
3. OPL-**CCL7**-LPM (**MCP-3**-AM-ShigaHIS; formerly designated OPL98109 in Table 6 of the application or *in vitro* data and OPL98103 for the *in vivo* data); and
4. OPL-**CXCL12**-LPM (**SDF-1β**-AM-Shiga; formerly designated OPL98103 in Table 6 of the application).

In some cases in the former nomenclature is employed. All were prepared as described in the application.

1. *IN VIVO* OPL-CXCL12-LPM STUDIES

Toxicity Study

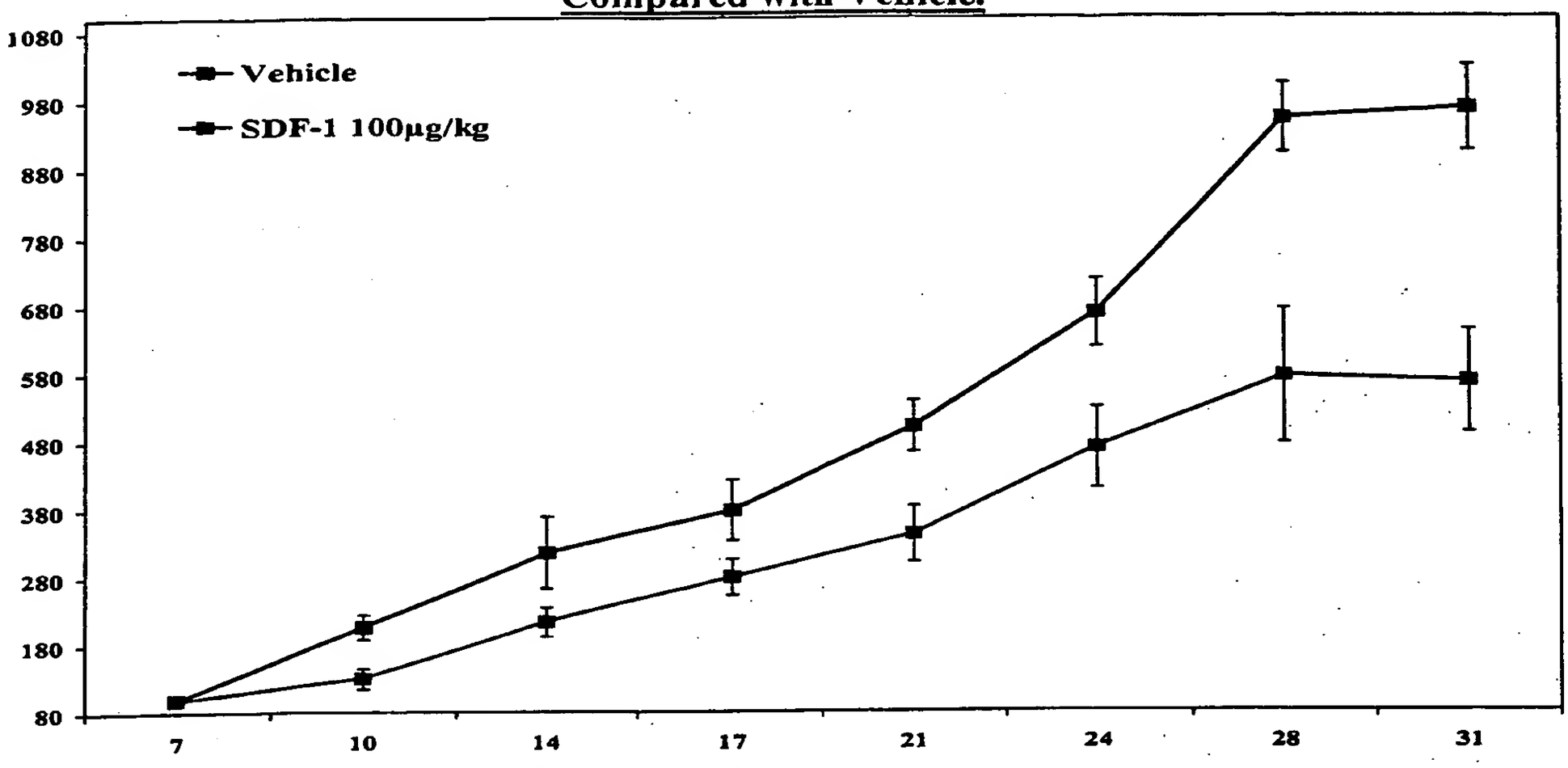
A 2mg/kg bolus of OPL-CXCL12-LPM was given to 5 Female and 5 Male Athymic Nude (nu/nu) mice (25 g) which were observed for 14 days. Gross necropsy examination revealed no organ damage and both control and test mice gained weight and were healthy. A previous experiment revealed no toxicity after examining the histopathology of stained tissue from several organs form control and test mice. Organs from this experiment have been stored with histopathology examination eminent. A previous toxicity study was reviewed elsewhere (McDonald, 2001; P435, Paragraph 2). In a total of 4 Xenograft 30 days plus studies with this molecule no animals have died due to test compounds toxicity.

MCF-7 Breast Cancer Cell Xenograft Model

A study was performed to evaluate the effects of OPL-CXCL12-LPM compared to Vehicle in an established tumor xenograft model. Female nude mice (nu/nu) were injected with 2.5 million cells (0.2 ml of PBS/Matrigel) of the estrogen dependent breast carcinoma cell line MCF-7. Intraperitoneal dosing began on day 7 and continued every day through day 21. Tumors were allowed to continue to grow until Day 31. Figure 2 shows a statistically significant decrease in the rate of MCF-7 tumor growth as measured by tumor volume with 100 μg/kg of OPL-CXCL12-LPM. The final tumor weights from test animals decreased by an average of 35% and final tumor volumes by 41.5% that of control (significant using $p < 0.05$ two tail t-test).

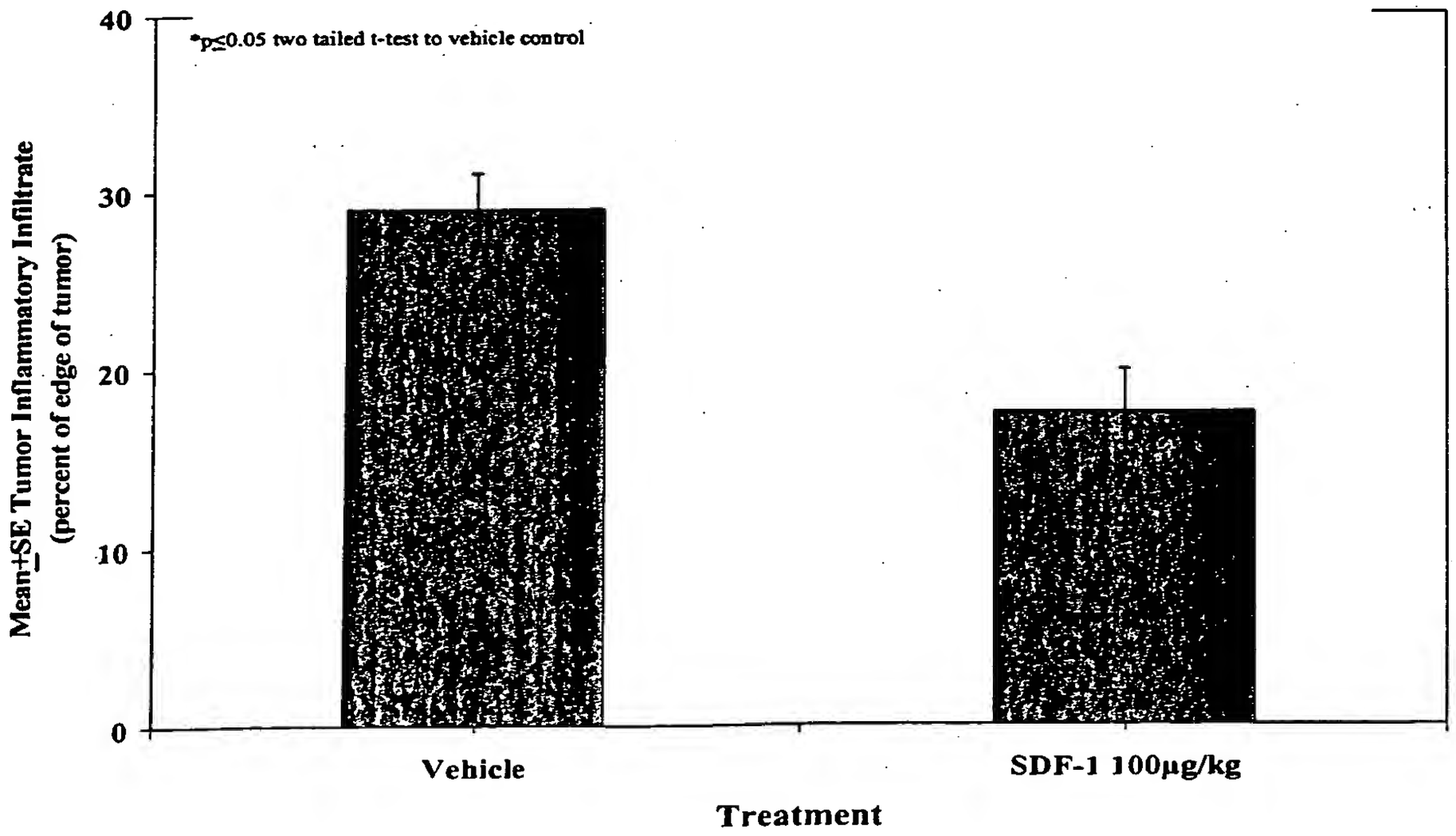
A clearer picture emerges upon histological examination, which allows visualization the extent of intratumoral necrosis and vacuolization (see Figure 4 below, anti-Ki-67 staining, Figure 4 and page 435 of McDonald, 2001). Apoptotic staining can be done to judge the numbers of dying cells.

Figure 2. Effects of Intraperitoneal OPL-CXCL12-LPM on MCF-7 Tumor Growth Compared with Vehicle.



The X axis is the measured volume of tumors(mm3). Here SDF-1 refers to OPL-CXCL12-LPM.

Figure 3. Effects of Intraperitoneal OPL-CXCL12-LPM on Inflammatory Infiltrate Around MCF-7 Tumors Compared with Vehicle



Here SDF-1 refers to OPL-CXCL12-LPM.

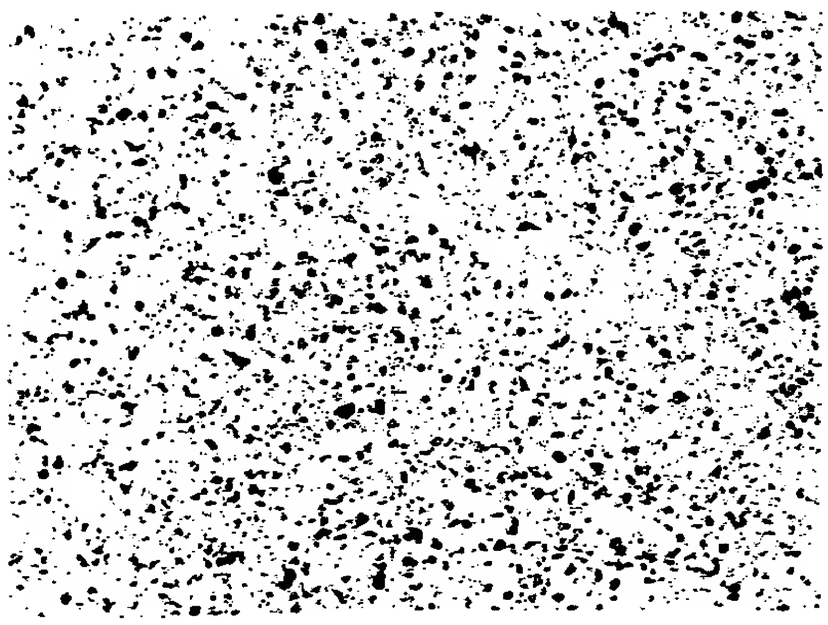
Figure 4. Histological Staining of Representative Tumors

Anti-CD31

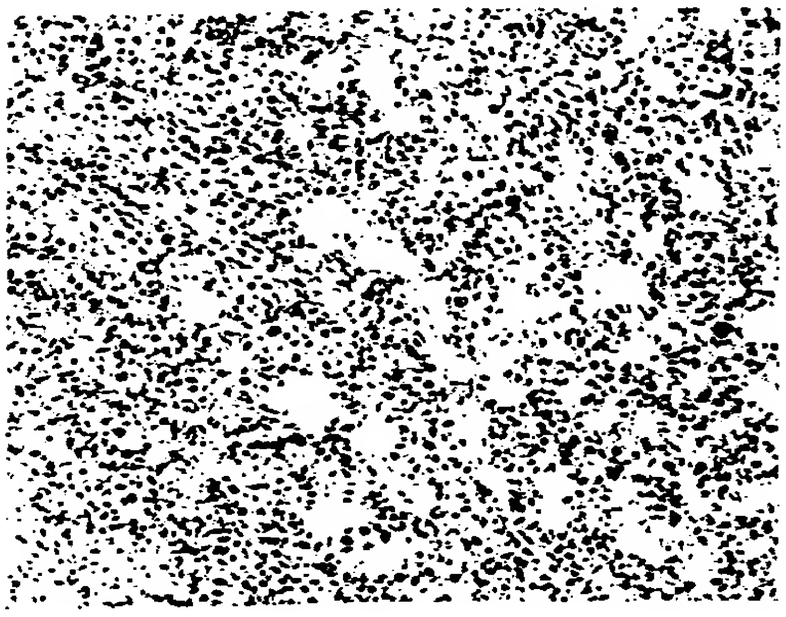
CONTROL

TEST

ANTI-Ki-67



CONTROL



TEST

Tumors were excised and exposed (along with peripheral tissue) to histological and microscopic examination as shown in Figure 3 and 4. Microscopic evaluation of leukocyte infiltrate around the periphery of the tumors showed that there was 36% less cells in the tissue of treated animals and was statistically significant (Figure 3). The tissue was processed 10 days after the last dose given to the mice. This suggests that most TAMs and infiltrating (migrating) leukocytes were destroyed by the drug during dosing.

CD-31 (PECAM-1) is a cell adhesion molecule from a family of closely related cell surface glycoproteins involved in cell-cell interactions during growth that are thought to play an important role in embryogenesis and development. CD31 is a glycoprotein expressed on the cell surfaces of monocytes, neutrophils, platelets and a subpopulation of T cells. It is also expressed on the surface of adult and embryonic endothelial cells. The CD-31 staining suggests that some leukocytes (circular staining) and angiogenesis (endothelial cells – elongated shaped staining) are present in the growing control tumors. Conversely there is no staining in the treated tumors suggesting the absence of macrophages (athymic mice have no T cells) and the absence of intratumoral endothelial cell blood vessels. This is consistent with

the mode of action of the compound which eradicates these activated cell types. These data support the conclusion that OPL-CXCL12-LPM eradicates intratumoral newly forming blood vessels as evidenced by the lack of cross sectioned (round white circles) vessels in treated tumors versus control tumors witnessed in a HT29 colon carcinoma xenograft study (McDonald, 2001; Figure 4, P 435).

The Ki-67 antigen is preferentially expressed during all active phase of the cell cycle (G_1, S, G_2 and M phases), but is absent in resting cells (G_0-phase). During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. This antigen is rapidly degraded as the cell enters the non-proliferative state, and there appears to be no expression of Ki-67 during DNA repair processes. A large number of cells appear to be proliferating in the non-treated tumors as shown by Ki-67 staining. In contrast treated tumors show little staining due to decreased tumor progression and it appears that many of the cancer cells have necrosed as evidenced by the clear vacuoles in the field.

This is the second MCF-7 breast cancer study showing efficacy and adds to the data set of two prior HT29 colon carcinoma cell xenograft studies with OPL-CXCL12-LPM. This compound has shown efficacy in 4 xenograft studies. Collectively, the data supports the view that the drug targets CXCR4 expressing tumor, endothelial and leukocyte cells as predicted and is proof of concept of specificity (no observed toxicity upon i.p. delivery) and efficacy *in vivo*. Additionally, there was no evidence of toxicity upon i.p. delivery.

2. *IN VITRO* OPL-CXCL12-LPM STUDIES

Tissue culture was performed using cell lines obtained from the ATCC or specific human cells enriched by gradient centrifugation from fresh blood obtained from human donors. Cell culture conditions were determined from the ATCC insert instructions and standard conditions for donated blood. Control and test conditions were carried out in quadruplicate and results shown as the Mean +/- SEM. In general cells were left for 24 h prior to treatment and the cell viability determined by the MTT color-dye assay or by Trypan Blue exclusion. This was the methodology for all compounds tested on such cells.

The compound routinely shows dose responsive toxicity to MCF-7 carcinoma cells used in the *in vivo* studies. In an earlier Declaration of record, *in vitro* dose response data on human U251 glioma, HT29 colon cancer and THP-1 (monocyte cell line) cells were provided. The data presented in Figures 5 and 6, below show OPL-CXCL12-LPM dose responses on freshly isolated human monocytes and T cells, respectively. The compound did

not show any toxicity toward human astrocytes (maximum of 40% cell death) or human foreskin fibroblasts until the dose reached 10 µg/ml and above. The compound showed non-dose response toxicity to human neurons with a maximum effect of 75 % killing at 10 µg/ml.

Figure 5.

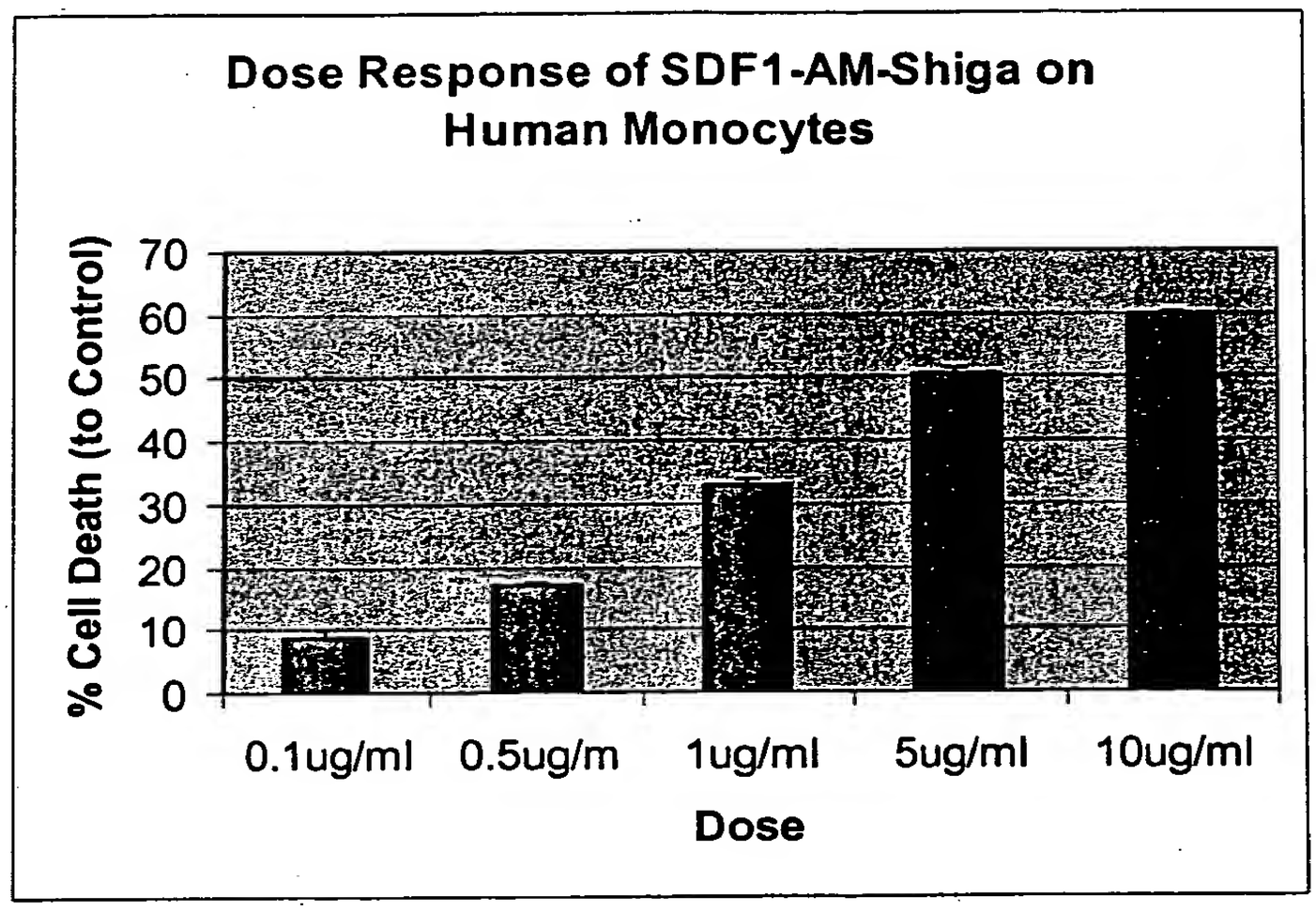
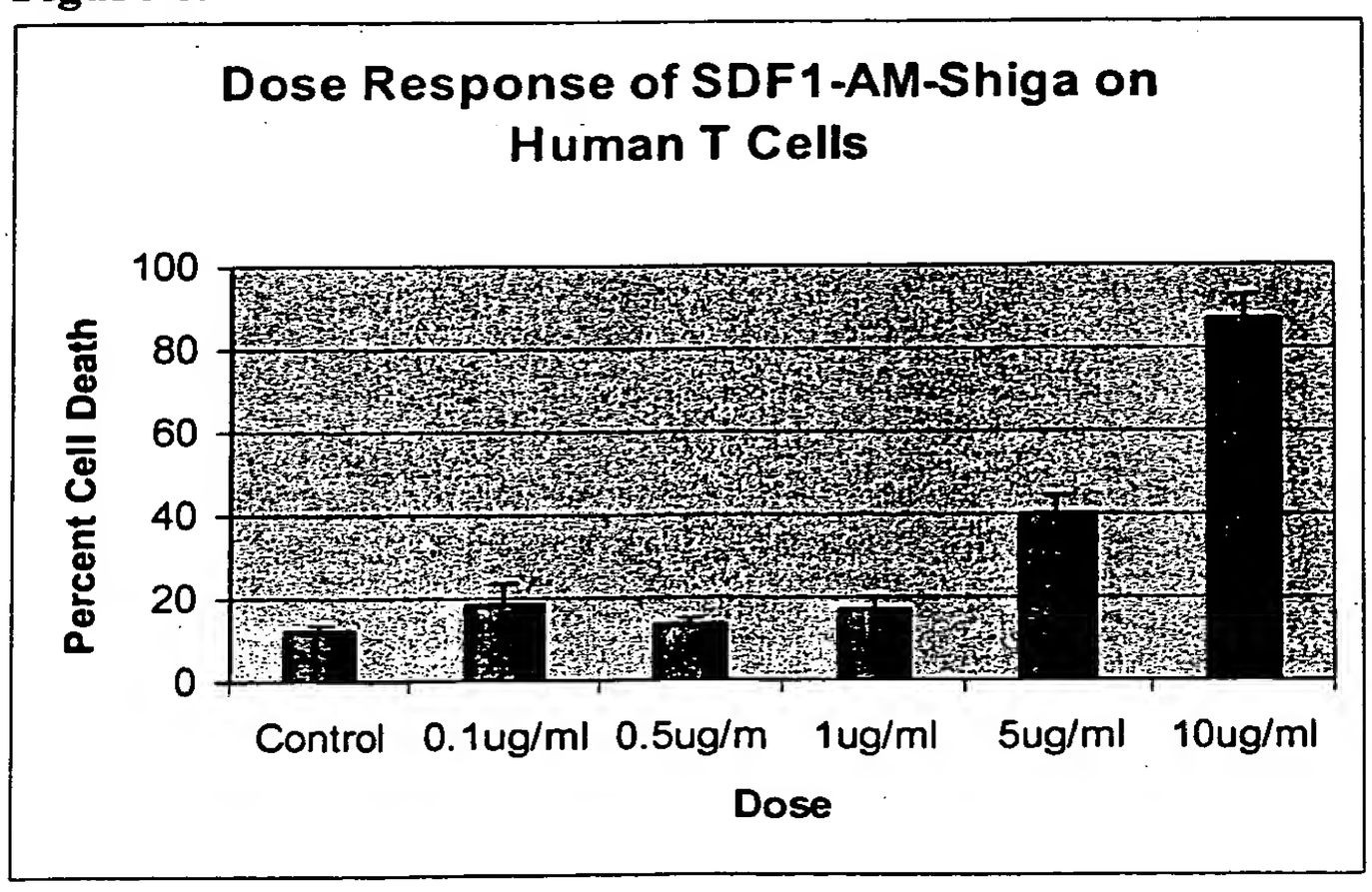


Figure 6.



One hundred percent killing is not observed on these freshly isolated cells, which could be explained if a saturating dose was not achieved. On the other hand, non-activated (non- targeted receptor expressing cells) are likely present and they would not succumb to the

drug. I have observed less than 100% killing using freshly isolated human cells on several occasions with the CCL2 and CXCL12 LPM variants. When using proliferating cells rather than freshly isolated cells, CCL2, CCL7 and CXCL12 have consistently shown 100% killing of highly activated proliferating THP-1 monocytes (cell line) at doses of approximately 20 µg/ml. Monocytes appear less susceptible to OPL-CXCL12-LPM than T cells, which is consistent with their lower expression of the targeted receptor CXCR4.

3. *IN VIVO* OPL-CCL2-LPM STUDIES

Toxicity Study

A 2mg/kg bolus of OPL-CCL2-LPM was given to 5 Female and 5 Male Athymic Nude (nu/nu) mice (25 g) which were observed for 14 days. Gross necropsy revealed no organ damage and test mice variations mirrored that of controls. Organs were retrieved and stored prior to ongoing histological examination. No animals died during the 31 day study due to toxicity of the compound. Activity of drug lots are routinely tested using MCF-7 breast carcinoma cells.

MCF-7 Breast Cancer Cell Xenograft Model

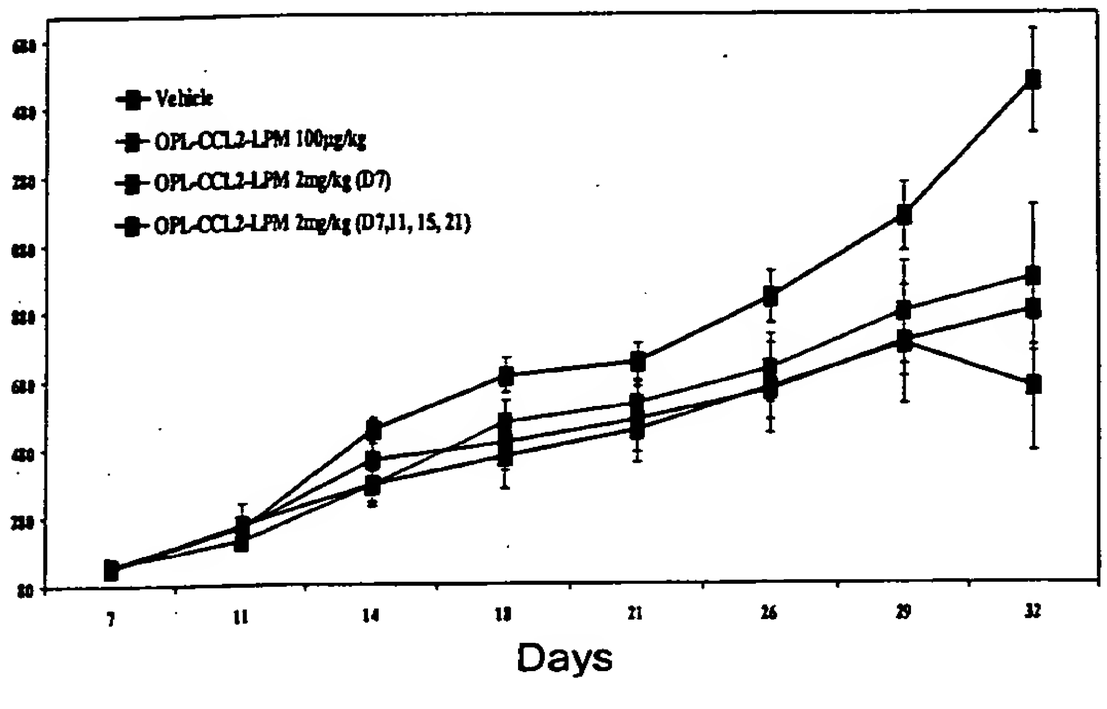
A study was performed to evaluate the effects of OPL-CCL2-LPM compared to vehicle in an established tumor xenograft model. Female nude mice (nu/nu) were injected with 2.5 million cells (0.2 ml of PBS/Matrigel) of the estrogen dependent breast carcinoma cell line MCF-7. Intraperitoneal dosing began on day 7 and cohorts received either vehicle; (1) one dose of 2 mg/Kg on day 7; (2) 2mg/Kg on days 7, 11, 15 and 21 or (3)100 µg/Kg every day from day 7 through day 21. Tumors were allowed to continue to grow until Day 32 (Figure 7). The percent change in body between different cohorts including the control did not exceed 0.5%. Treatment induced a statistically significant decrease in the MCF-7 tumor growth as measured by tumor volume and weight. The final tumor weights from groups 1-3 decreased by 41, 58.6 and 36% that of control (significant using $p < 0.05$ two tail t-test). The final tumor volumes from groups 1-3 decreased by 47, 63 and 40.4% that of control (significant using $p < 0.05$ two tail t-test). Organs and tumors have been collected for histopathology (data not shown). This study indicates that a single or minimal repeated dosing is enough to at significantly decrease the rate of tumor growth. MCP-1 is known to target receptors on these cancer cells and infiltrating macrophages (see, *e.g.,* Youngs *et al.* (1997) *Int. J. Cancer 71*:257-266) and OPL-**CCL2**-LPM is a conjugate that contains **MCP-1**. In the light of the *in vitro* data with this compound and the OPL-CXCL12 –LPM histological

data indicating macrophage eradication, OPL-CCL2-LPM appears to exhibit similar effects.

4. *IN VITRO* OPL-CCL2-LPM STUDIES

OPL-CCL2-LPM/ MCP-1-AM-Shiga dose responsive cytotoxic activity has been demonstrated on THP-1 human monocytes and human MCF-7 breast carcinoma cells. A dose response of cytotoxicity on THP-1 cells in tissue culture is shown in Figure 8. The compound has been shown to kill freshly isolated T cells and monocytes from human healthy donors (data not shown) and human U937 monocytes (Figure 8, bottom row) in a dose responsive manner. OPL-CCL2-LPM shows no activity on murine P388D1 monocytes which do not express the mCCR2 receptor (Boring, 1996) or human U251 glioma (astrocytes) cells. In one set of experiments no activity was detected on primary human neurons and human astrocytes or in W1095HF and W1093NMA, two human astrocytes cell lines.

Figure 7. Effects of Intraperitoneal OPL-CCL2-LPM on MCF-7 Tumor Growth Compared with Vehicle.



The X axis is the measured volume of tumors (mm3).

The above *in vitro* and *in vivo* studies confirm the targeting activity of OPL-CCL2-LPM on CCR2 receptor-bearing cells. As known in the literature, see above discussion, MCF-7, endothelial cells and macrophages all express this receptor.

5. *IN VITRO* OPL-CCL11-LPM STUDIES

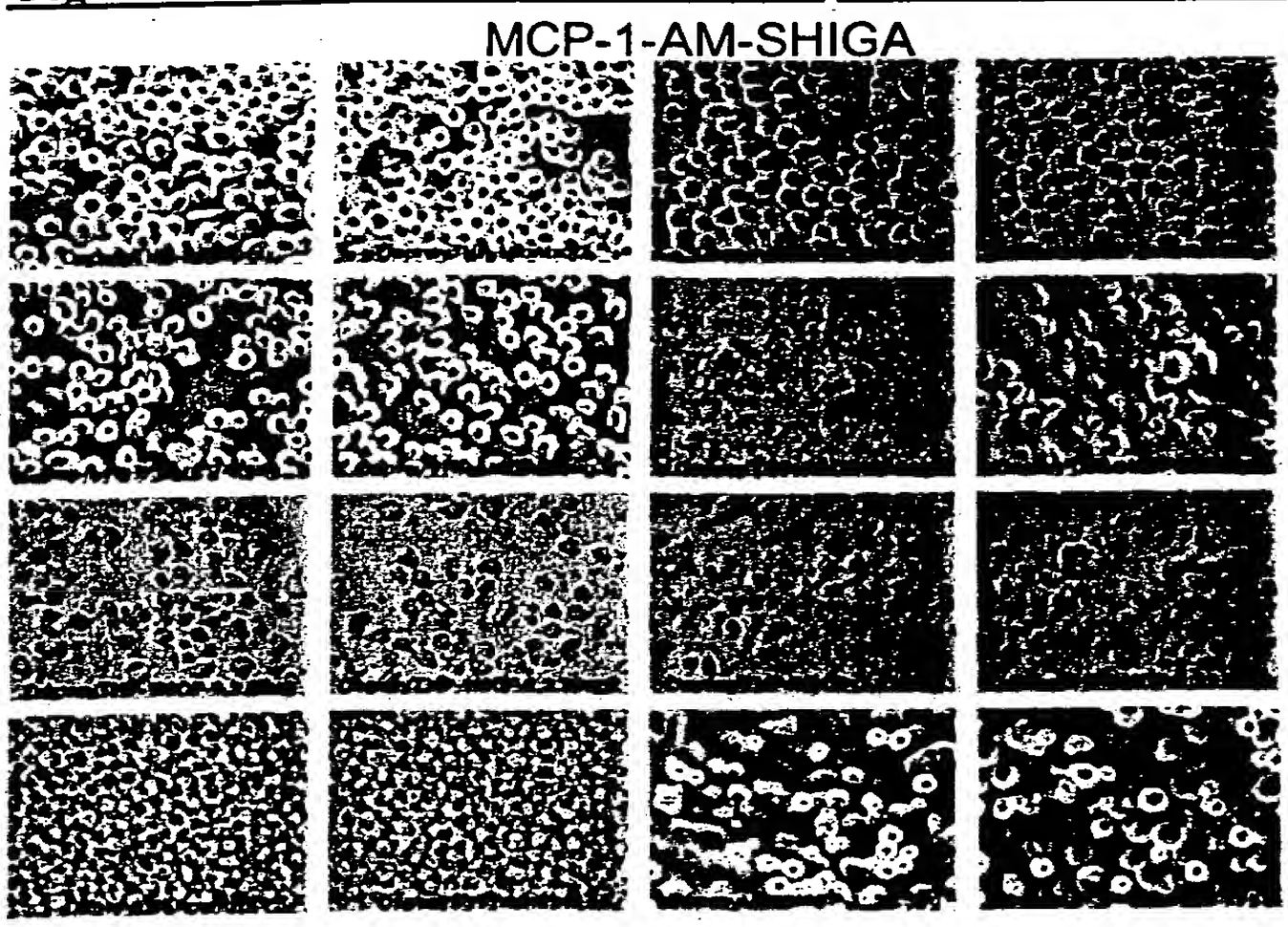
The cell isolation and culture conditions were the same as for the CXCL12 variant studies. The activity of freshly purified OPL-CCL11-LPM/Eotaxin-AM-Shiga conjugate, which targets the CCR3 receptor (see, *e.g.*, Table 1 in the application) was studied. Serial dilutions of the product were made and added to human T cells and monocytes (Figures 9 and

10). Both these cell types are known to express the targeted CCR3 receptor Significant cell killing was achieved at high dilutions and there was a demonstrated dose response.

6. *IN VITRO* OPL-CCL7-LPM STUDIES

THP-1 monocytes were cultured as described above. A small batch of the MCP-3-AM-Shiga conjugate was made and 10 μg/ml was added to wells in quadruplicate (see Figure 12). The second and third frames of Figure 11 show different fields of a representative culture well containing THP-1 cell survivors in suspension. As described in the application MCP-3 binds to CCR1, 2, 3 and 5, which occur on these cells. The results show that there are many necrosing cells, sick cells and cellular debris. At this concentration 100% cell death was achieved.

Figure 8. Activity of OPL-CCL2-LPM on THP-1 and U937 Monocyte Cell Lines.



Reading left to right from the top there are duplicates of THP-1 control wells followed by increasing doses of compound to a maximum of 22.5 (last 2 wells third row). On the fourth row there are 2 wells of U937 monocyte controls and then 2 wells of 22.5 μg/ml of compound added. Quite clearly OPL-CCL2-LPM has a dramatic killing effect on both these cell isolates.

Figure 9.

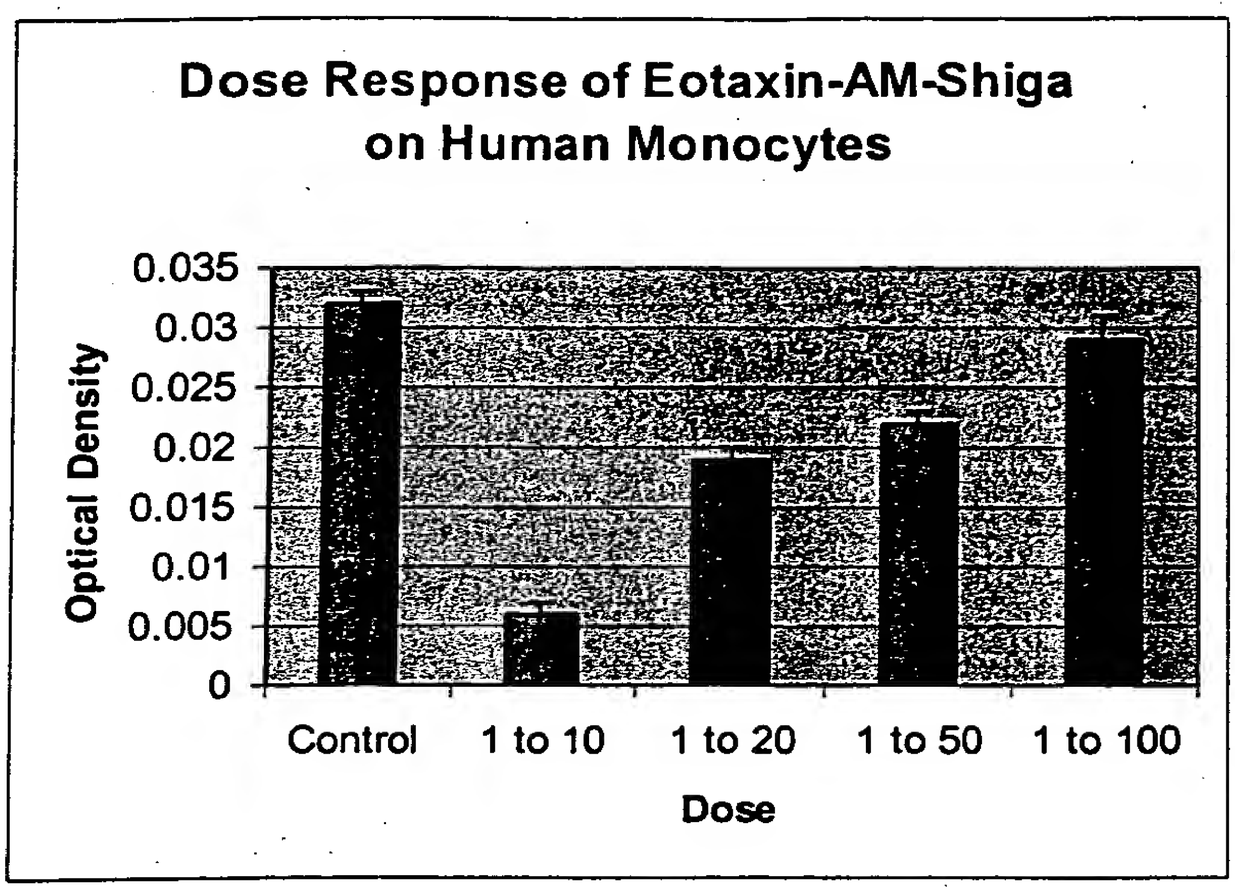


Figure 10.

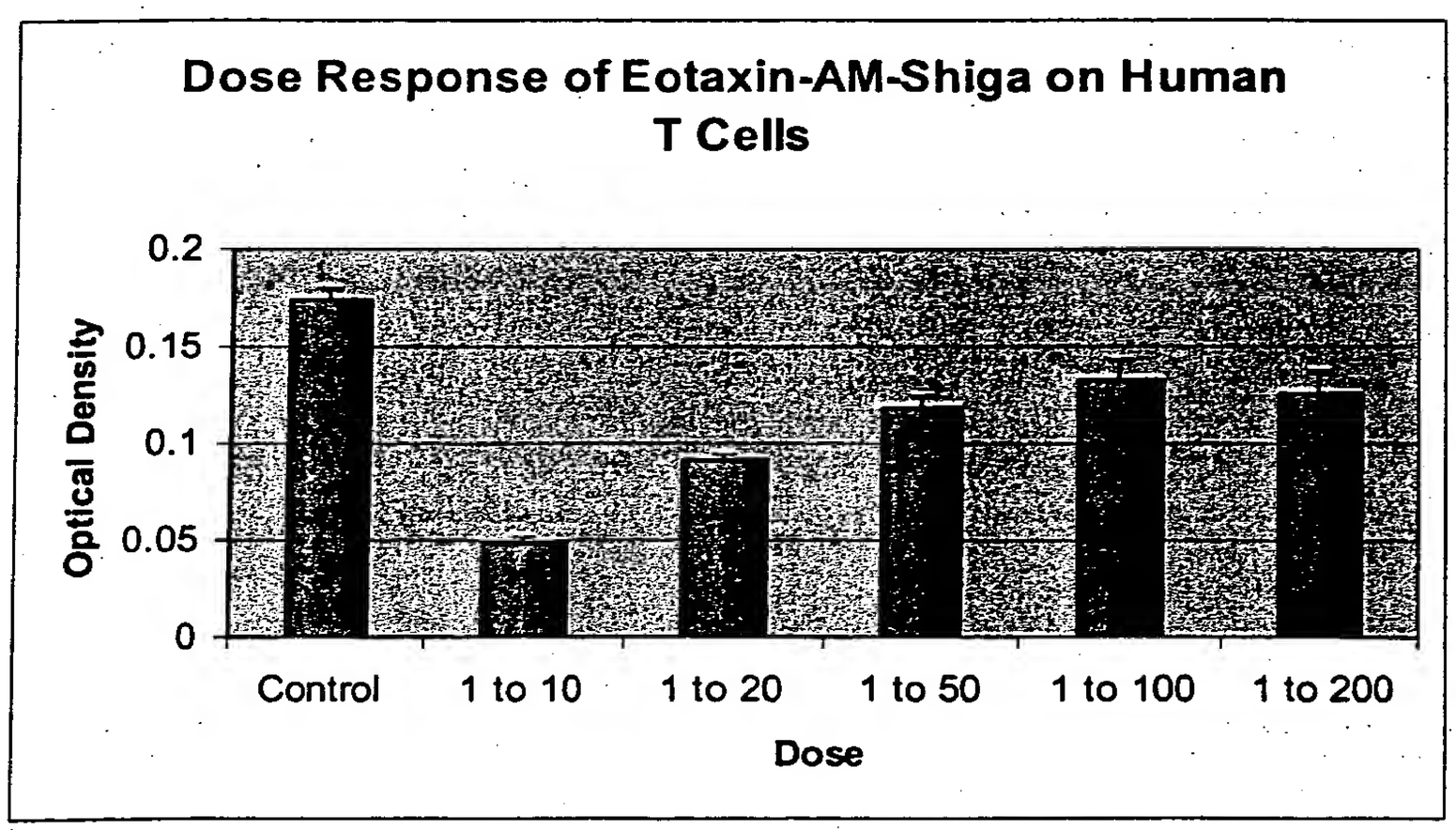
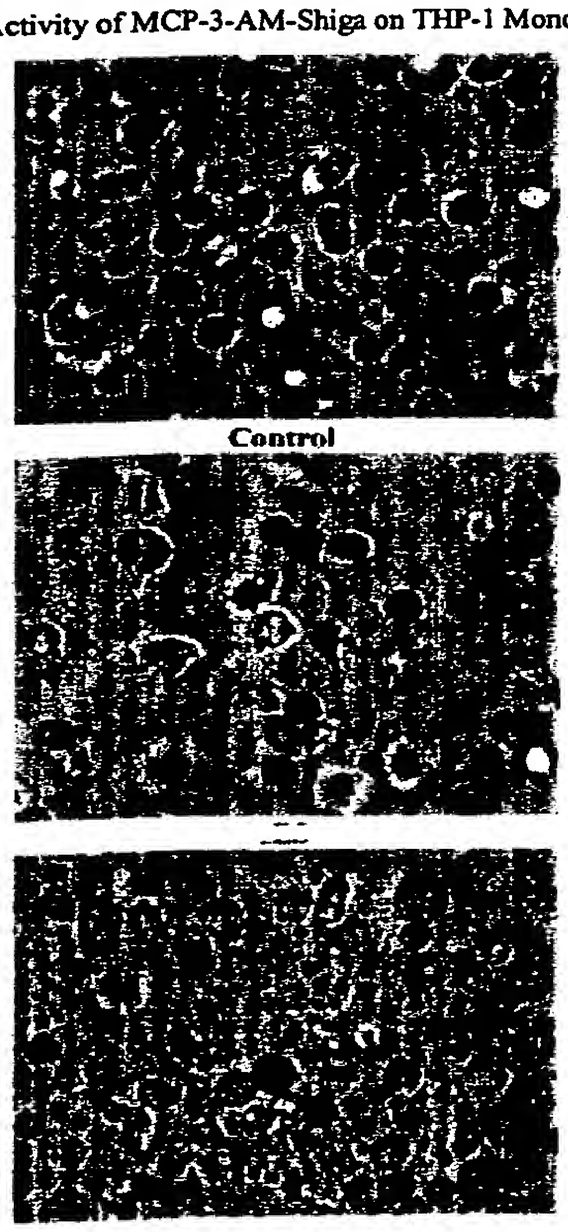


Figure 11. Activity of OPL-CCL7-LPM of THP-1 cells



7. SUMMARY AND CONCLUSION

In this DECLARATION daa is provided for conjugates containing SDF-1β, Eotaxin, MCP-3 and MCP-1. *In vitro* and *in vivo* data are provided and show that each conjugate targets receptors on cells as described in the application. The results show that the LMPs prepared and described in the application exhibit toxicity toward targeted cells. The results also evidence cell selectivity. The *in vivo* studies clearly show that there is little, if any, toxicity. This is as expected and as described in the application as LPMs are designed to target activated chemokine receptor expressing target cells involved in inflammatory processes. We have shown that four different LPMs can target leukocytes in accord with the

specificities of the chemokine targeting portion and that the LPMs can deplete and/or inhibit proliferation of targeted cells.

C. Evidence that targeting leukocytes is an effective modality

This section lists the mechanisms of action and other properties of FDA approved Immunosuppressive and Cell-Depleting drugs. This evidences that there are numerous approved drugs that target leukocytes; reduction in their amounts is a recognized mode of treatment of numerous diseases.

1. Immunosuppressives

Immunosuppressive drugs for the treatment of leukocyte-mediated inflammatory diseases which have been around for at least 30 years and longer . Some drugs have superseded these immunosuppressives, but in serious cases or where there are no alternatives they are still used. A problem with these drugs is that they are too pleitropic, can have serious side effects and are non-selective. This led researchers in the quest for more selective therapeutics. Some examples of immunosuppressives, their mechanism of action and side effects are given below.

a. Corticosteroids

- **Action:** Wide range of immunosuppressive effects including, lymphocytopenia, monocytopenia (including eosinophils), reduction in monocytic proteases, and blockage of chemotactic factors.
- **Indications:** Transplant Rejection, CNS trauma, Pulmonary, Kidney and Dermatological Diseases, Acute MS.
- **Side Effects:** Cushing's syndrome Hypothyroidism, Hypertension, Lymphocytopenia, Gastrointestinal ulcers, Infections, Dermatological diseases, Cataracts, Osteoporosis.

b. Cyclophosphamide and Chlorambucil

- **Action:** These alkylating agents cross link DNA and RNA and therefore inhibit leukocyte proliferation.
- **Indications:** Various Cancers, Rheumatic and Kidney diseases, SLE, Interstitial Lung diseases, Vasculitides.
- **Side Effects:** Teratogenic, Gonadal, Hematologic, Pulmonary, Kidney and Bladder toxicity, Malignancies.

c. Cyclosporine

- **Action:** Inhibits cytokine production including IL-2 (a T cell activator and mitogen) by activated T cells via calcineurin phosphatase inhibition.
- **Indications:** Transplant Rejection, Arthritic, Kidney and Dermatological diseases, IBD.
- **Side Effects:** Hepatotoxic, Neurotoxic, Nephrotoxic, Hypertension, Infection, Malignancies, Osteoporosis.

d. Novantrone

- **Action:** A DNA-reactive agent that intercalates into and crosslinks with DNA and RNA. It is also a potent inhibitor of topoisomerase II, an enzyme responsible for uncoiling and repairing damaged DNA. It has a cytocidal effect on both proliferating and nonproliferating cultured human cells, suggesting lack of cell cycle phase specificity. It also inhibits B cell, T cell, and macrophage proliferation and impairs antigen presentation, as well as the secretion of IFN-γ, TNF-α, and IL-2.
- **Indications:** Cancers, Secondary Progressive MS.
- **Side Effects:** Malignancies, Leukopenia, Myelosuppression, Renal Failure, Congestive Heart Failure, Interstitial Pneumonitis, Dermatological reactions.

2. Cell-Depleting Agents

Depletion of macrophages with chloroquine, colchicine and diphosphonates in models of spinal cord injury and multiple sclerosis showed efficacy and thus proof of that macrophages are the culprits of secondary tissue damage and ultimately the presentation of disease/trauma (Guilian papers). Unfortunately these agents cannot be used in the clinic for these purposes. Other targeting agents are in the clinic or are FDA approved. These cell targeting agents, especially lytic antibodies have been used to eliminate mostly cancerous leukocytes or cancers themselves. Most have proven to be too pleiotropic and can have severe side effects or have shown to be effective for a small percentage of patients. Ligand-fusion toxin proteins usually possess cell-targeting antibodies or pleiotropic cytokines (e.g., IL-2, IL-3, and IL-4) and have toxicity issues. These compounds are mostly used in the cancer field since toxicity is weighed against life extension for the patient. Using these particular classes of ligand to target cells has by enlarge been disappointing because of the side effects. As demonstrated previously, targeting the chemokine system, as described in the instant application, is far less toxic.

Cell-depleting agents, which the instantly claimed conjugates are, are known to be effective for treatment of diseases with an inflammatory component. Examples of cell-depleting agents include:

a. Campath

- **Action:** Alemtuzumab is an anti-CD52 recombinant humanized monoclonal antibody, which targets a CD52, which occurs on the surface of many normal and cancerous leukocyte subtypes. Cell lysis occurs via complement-dependent cytotoxicity (CDC) and antibody-dependent cell mediated cytotoxicity (ADCC).
- **Indications:** B-cell Chronic Lymphocytic Leukemia (B-CLL)…
- **Side Effects:** Pancytopenia/Marrow Hypoplasia, Autoimmune Idiopathic Thrombocytopenia, Autoimmune Hemolytic Anemia, Severe Infusion Reactions and Infections affecting several organs. Fatalities have occurred from these listed effects. Although Campath showed efficacy in MS trials, its high toxicity recently caused cases of idiopathic thrombocytopenic purpura with one fatality and three other deaths from other side effects (Reuters, Sept. 16th 2005).

b. Mylotarg

- **Action:** Gemtuzumab-ozogamicin is a ligand-toxin fusion protein composed of a humanized monoclonal antibody against CD33 fused to a calcheamicin derivative. Once endocytosed by target cells the toxin (an enediyne) causes DNA breakage and the cells die apoptotically.
- **Indications:** Acute Myeloid Leukemia.
- **Side Effects:** Anemia, Neutropenia, Thrombocytopenia, Myelosuppression, Infections, Bleeding, Hepatotoxicity, Mucositis.

c. Ontak

- **Action:** Denileukin diftitox is a ligand-toxin fusion protein composed of the human IL-2 ligand fused to a genetically modified version of diphtheria toxin. IL2 receptor bearing cells including T cells take up the protein and are killed by the ADP ribosylation of elongation factor 2 which terminates cellular protein synthesis.
- **Indications:** Cutaneous T Cell Lymphoma. In clinical trials for GVDH, Psoriasis, non-Hodgkin's Lymphoma, Chronic Lymphocytic Leukemia.
- **Side Effects:** Hypersensitivity Reactions, Immunogenicity, Vascular Leak Syndrome.

d. Rituxan

- **Action:** Rituximab is a chimeric (mouse/human) anti-CD20 antibody. The Fab domain of Rituximab binds to the CD20 antigen on B lymphocytes, and the Fc domain recruits immune effector functions to mediate B-cell lysis in vitro. Possible mechanisms of cell lysis include CDC and ADCC.
- **Indications:** Relapsing, Refractory and Follicular B cell NHL.

- **Side Effects:** Cytopenias similar to Bexxar, Severe Infusion and Hypersensitivity reactions (Pulmonary and Cardiovascular effects). Renal toxicity, Infections. Female gender, Pulmonary infiltrates, Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma were more frequently associated with fatal outcomes than other reported side effects.

e. Zenapax

- **Action:** Daclizumab is an immunosuppressive humanized monoclonal antibody which specifically binds the CD25 alpha subunit of the human IL-2 receptor. This receptor is blocked on human activated T cells and causes there deactivation. The antibody inhibits cell cycle progression and induces T cell apoptosis.
- **Indications:** Transplant rejection, Cancer.
- **Side Effects:** Severe Infections, Death (4 drugs co-treatment), Cardiovascular, Gastrointestinal and Hematalogic reactions, Hypersensitivity, Anaphylaxsis.

f. Zevalin

- **Action:** Ibritumomab-tiuxetan-Y^{90} is a ligand-toxin fusion protein comprised of a murine monoclonal antibody (ibritumomab) bound to a radioactive isotope (yttrium-90) by a strong linking agent (tiuxetan). The antibody targets the CD20 antigen on the surface of mature B cells and B-cell tumors, (and not CD20-negative progenitor cells). The radioactivity kills the cells.
- **Indications:** Relapsed or Refractory low-grade, Follicular or Transformed B-cell NHL.
- **Side Effects:** Severe Cytopenias and Infusion Reactions, Hypersensitivity (Cardiovascular and Pulmonary systems can be fatal), Severe Mucocutaneous reactions (some fatal), Myeloid Malignancies.

* * *

Applicant : John R. McDonald et al.
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Filed : July 22, 1999
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Attorney's Docket No.: 17080-002002/601B

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

John R. McDonald

JOHN R. McDONALD

Date: 02/09/06

17080-002002/601B